

## THE AXIAL GRADIENTS IN HYDROZOA.

### VII. MODIFICATION OF DEVELOPMENT THROUGH DIFFERENTIAL SUSCEPTIBILITY.

C. M. CHILD.

From the Hull Zoölogical Laboratory, the University of Chicago.

In the preceding paper of this series the physiological analysis of embryonic development in certain species of hydrozoa was begun with a study of the physiological gradients and their changes during the usual course of development (Child, '25). The present paper is a continuation of this analysis and is concerned with the modification of development resulting from the differential susceptibility to various agents of different levels of the polar gradient or gradients.

#### MATERIAL AND METHOD.

Developmental stages of the leptomedusa, *Phialidium gregarium*, abundant in Puget Sound, constituted the chief experimental material. Since the medusæ shed eggs or sperm more or less continuously early developmental stages are readily obtained by keeping the sexually mature medusæ in large containers for a time and then collecting the embryonic stages from the bottom. Developmental material obtained in this way shows a certain range of stages, depending on the length of time the medusæ are kept in the container, but by using large numbers of medusæ sufficient material for one or more experimental series can usually be obtained within a few hours. With this procedure the stages range from newly fertilized eggs to more or less advanced cleavage or early blastulæ and still greater uniformity can of course be obtained by sorting the stages, but this is unnecessary for most purposes. A few experiments were performed with the early planulæ of *Gonothyraea clarkii* after their emergence from the gonophores.

The experimental procedure consisted in the exposure of the developmental stages to concentrations of agents which had been

found by preliminary experiment to inhibit development to a greater or less degree but not to be directly lethal. Agents and indicated concentrations used are as follows: KCN, *m*/20000, *m*/25000, *m*/50000; HCl, pH 6.8 —, pH 7.2, pH 7.3, pH 7.4 +;<sup>1</sup> LiCl, *m*/25, *m*/50, *m* 80, *m*/160; ethyl urethane, *m*/25 and very dilute neutral red. All solutions were made up in sea water and the concentrations given are those indicated by amount of agent and volume of water used. For experiment finger bowls holding some 400 cc. were used. These could be brought under a binocular or even under low power of a compound microscope without removal or disturbance of the developing material. When volatile agents were used bowls with an edge giving good contact with a glass plate were selected, being completely filled with the solution and covered with the plate so as to exclude air. Atmospheric humidity being high, there was practically no loss of water under these conditions and certainly no appreciable loss of the agent since renewal was usually made daily.

The variations in experimental procedure may be grouped as follows: (a) continuous exposure to a certain concentration beginning with the earliest stages obtainable; (b) temporary exposure to a certain concentration beginning with the earliest stages, followed by lower concentration or by return to sea water; (c) early development in sea water, exposure to agent beginning at later stage (blastula, early planula).

The developmental stages of *Phialidium* are so susceptible to change in condition that some degree of developmental modification frequently occurs in sea water in the laboratory if conditions are not maintained near the optimum. For example, if the developmental stages remain too long in the container with large numbers of medusæ, differential inhibition of development results, the modifications being similar to those induced by other means. Under these conditions there is a decrease in pH, resulting chiefly from the CO<sub>2</sub> production of the medusæ. There may also be some accumulation of other metabolic products and

<sup>1</sup> At the time these experiments were performed it was believed that the increase in H-ion concentration was the chief inhibiting factor in the acid experiments, but in the light of the recent work of Smith and Clowes ('24) it appears probable that the increase in CO<sub>2</sub> resulting from the addition of HCl to sea water is more important than the increase in H-ion concentration. In my experiments no attempt was made to determine CO<sub>2</sub> tension after addition of HCl.

some decrease in oxygen content. When development takes place in standing water several centimeters deep without renewal some degree of differential inhibition and modification usually occurs at the bottom while attachment and development of hydranth-stem axes proceed in the normal manner (Child, '25) on the sides of the container near the surface. Here also there is some decrease in pH at the bottom. When development takes place in a layer of water only a few millimeters deep no such differences appear and development is wholly or almost wholly normal if the material is otherwise in good condition. Occasional cases of modification usually occur even in the best material kept as nearly as possible under optimum laboratory conditions. Such modifications doubtless result from factors affecting particular eggs or spermatozoa before development or from poor condition of certain individuals.

Experiments with the medusæ as the inhibiting agents were performed by placing a number of the medusæ in small aquaria or bowls with the developmental stages and determining the pH colorimetrically from time to time.<sup>1</sup> The modifications produced by crowding, standing water etc., are exactly similar in character to those produced by the various other agents used. Some forty years ago Metschnikoff ('86) described as variations similar modifications in the development of *Mitrocoma*.

As will appear below, with the various agents and conditions used, developmental modifications result from the differential susceptibility of different body levels. All agents and conditions used inhibit development, the higher levels of the gradient being most inhibited. In the lower concentrations differential acclimation, or after return to sea water differential recovery may occur, the higher levels of the gradients acclimating or recovering more rapidly or more completely than the lower levels, as indicated by increased developmental activity.

<sup>1</sup> The work of Smith and Clowes ('24) makes it probable that in these experiments, as in those with HCl (see footnote, p. 177) the chief inhibiting factor is the CO<sub>2</sub> resulting from the respiration of the medusæ, rather than the increase in H-ion concentration brought about by the dissociation of carbonic acid formed from a part of the CO<sub>2</sub>. The fact that the decrease in pH is rapidly reversed on shaking the water with air indicates that CO<sub>2</sub> production is responsible for it, but it is possible that other products of medusa metabolism may play some part in the inhibition of development.

DIFFERENTIAL INHIBITION IN THE DEVELOPMENT OF  
THE PLANULA.

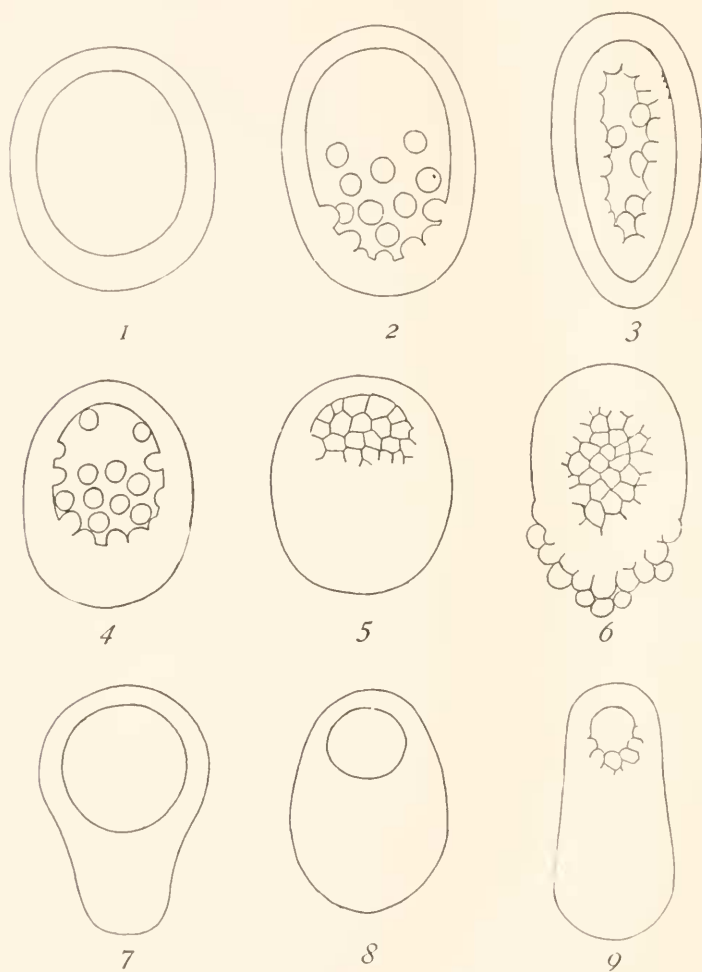
It is important to note first of all that there is no evidence of any specific effect on development of any agent used. The relations of the differential modifications to the gradient are similar for all agents, though the degree of differential action differs for different concentrations and for different agents, being greatest for LiCl, *i.e.*, in LiCl the high end of a gradient is relatively more, the low end relatively less inhibited than with other agents used. The high degree of differential action of lithium salts has also been noted by MacArthur ('24) in the modification of echinoderm development.

Since the modifications produced by the different agents are similar, detailed statements of the results obtained with each agent would involve much repetition and are omitted for the sake of brevity. The various degrees of modification are briefly described and data concerning their occurrence with different concentrations and agents are given.

*The Lesser Degrees of Differential Inhibition.*— In concentrations somewhat above those used for developmental modification the cleavage stages often separate into single blastomeres or collapse into irregular cell masses which sooner or later die, but in the concentrations used for modification of later stages, cleavage proceeds without appreciable alteration. Susceptibility is found to increase during early development and the lower susceptibility of cleavage stages as compared with later stages, together with the fact that more or less time is necessary for the inhibiting action to become evident, undoubtedly account for the absence of modification of cleavage with the concentrations used.

Figs. 1, 2 and 3 are diagrammatic outlines of blastula, immigration stage, and early planula as they appear under good conditions in the laboratory, and represent approximately "normal" development. Figs. 4-9 show the less extreme degrees of differential inhibition. These modifications are in general more frequent in material exposed to the lower concentrations from the beginning of development or to the higher concentrations from advanced cleavage or early blastula. They consist in different degrees of increase and extension of the basal thickening of the

blastula wall and of all degrees of reduction, even to complete obliteration, of the blastocœl by continued mass ingrowth of the thicker parts of the wall instead of, or in addition to immigration



of single cells. The thickened region is a solid cell mass, usually without distinct boundary between ectoderm and entoderm. Fig. 6 represents a condition observed thus far chiefly in LiCl in which the blastocœl is completely obliterated by cells and basal emigration is occurring.

In all these modified forms development is retarded and locomotion is much slower than normal. Many of these in-

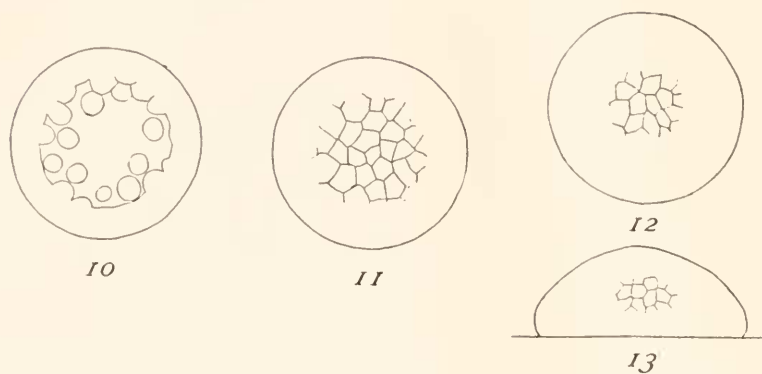
hibited forms advance slowly in contact with the bottom, being unable to support themselves free in the water, others revolve with little or no advance and some show reversal in the direction of locomotion (Figs. 6, 8). In these reversal of the susceptibility gradient and the reduction gradient (Child, '25) has also been observed.

These modifications represent an extension of basal characteristics and behavior toward the apical end, *i.e.*, a change of the more apical levels to the condition and behavior of the basal levels. The least degree of such modification consists merely in an increase of the basal thickening, an extension of the region of immigration toward the apical end and an increase in number of immigrating cells (Fig. 4). All gradations occur between this condition and forms in which almost the whole of the blastula wall is involved in immigration and ingrowth (Figs. 8, 9) and in some cases the cells of the basal region become so altered that they emigrate instead of immigrating (Fig. 6).

The differentially inhibited planulae which result from earlier stages like Figures 4-9 are more or less elongated forms with excess of entoderm, often completely solid. Since they are usually not sufficiently active to swim free, locomotion takes place in contact with the bottom, in most cases with apical end in advance, but in some reversed, as noted above. As ciliary activity decreases these larva do not attach by the original apical end, but simply come to rest gradually with some meridian of the longitudinal surface in contact and more or less flattening occurs along this meridian. In the absence of differential acclimation or recovery development may go no further, or one or more stolon axes may develop slowly. In any case the body becomes enclosed in a delicate perisarcial secretion.

*The More Extreme Degrees of Differential Inhibition.*—These appear more frequently in the higher concentrations or in material subjected to inhibiting action from the beginning of development. They constitute a continuous series with the forms already described and are separated only for convenience. In material exposed to sufficiently high concentrations or degrees of action from the early cleavage stages, elongation of the blastula does not occur, the whole wall remains thick and immigration and mass ingrowth occur in all regions, resulting in a spherical solid

larva, usually without distinct boundary between ectoderm and entoderm. Figure 10 shows an early stage, Figs. 11 and 12 show later stages of these forms.



When exposure to inhibiting agents is begun only after the blastula stage is attained, polar elongation, basal thickening and immigration may occur in the agent. In the higher concentrations, however, immigration or thickening of the blastula wall gradually extends apically and may involve all regions of the wall, the blastocœl may be obliterated and the elongation may disappear completely, the extreme modification short of death being spherical solid, apparently completely apolar forms indistinguishable from those described above (Figs. 11, 12). In these cases there is actual regression so far as visible axial differences, elongation and basal thickening are concerned.

These spherical solid larvæ, whether they arise from earlier stages before elongation occurs, or from later elongated stages by regressive changes, are, so far as can be determined, apolar. Susceptibility gradient and reduction gradient are no longer present. They have lost the capacity for definitely directed locomotion and roll about on the bottom, being usually unable to support themselves free in the water. They show no definite attachment reaction, but gradually become quiescent and after movement ceases adhere by some part of the surface and become more or less flattened (Fig. 13). If conditions do not permit acclimation or recovery, no further development occurs, though they may live for two weeks or more. In short, the physiological differences constituting polarity have apparently been obliterated



and the pattern is no longer axiate, but merely surface-interior (Child, '24, pp. 57, 93).

#### DIFFERENTIAL ACCLIMATION AND DIFFERENTIAL RECOVERY.

Under "normal" conditions the blastula develops into the elongated free-swimming planula (Fig. 14), which shows at first an apico-basal gradient, but later develops at the basal end a second gradient opposite in direction to the first, as described in the preceding paper (Child, '25). Later the planula attaches itself by the original apical end and the hydranth-stem axis develops from the original basal end, *i.e.*, from the secondary gradient (Fig. 15). As pointed out in the preceding paper, the hydranth-stem axis represents a new polarity arising by a process of budding from the original basal end of the planula.

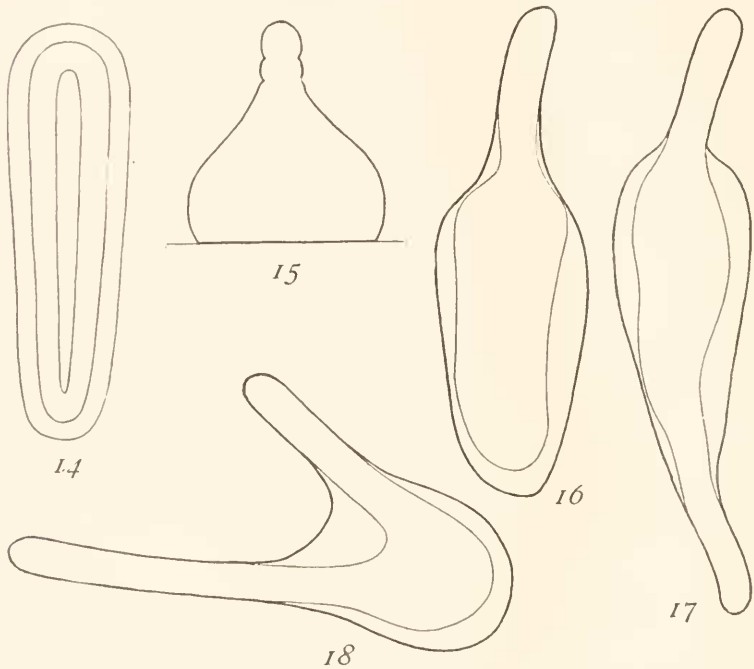
In the lower concentrations of agents which produce at first some degree of differential inhibition without completely obliterating polarity, differential acclimation or acquirement of tolerance may gradually occur in the course of a few days with further modification of development. Similarly differential recovery may occur on return to well aerated sea water after temporary exposure to concentrations which produce differential inhibition. Within a certain range of differential inhibition the regions most inhibited acclimate or recover most rapidly or most completely, as is indicated by further development. Since differential acclimation and differential recovery are secondary changes which take place following a differential inhibiting action, it is evident that they can modify only the later stages of development.

The first indication of differential acclimation or differential recovery in differentially inhibited planulae which still retain some degree of polarity is the outgrowth of a stolon from one or both ends. Fig. 16, apical stolon, and Fig. 17, apical and basal stolons, as seen from above, show characteristic forms. In the case of Fig. 18 the larva probably came to rest with the body bent upon itself and gave rise to a stolon from each end, though it is possible that one or both of these stolons represent new polarities. In these and later figures the heavy line indicates perisarc, the light line the outer surface of the cœnosarc.

As I have pointed out in an earlier paper (Child, '23), hydroid stolons represent somewhat inhibited physiological gradients or



reduced polarities and hydranth-stem axes can be easily transformed into stolons by inhibiting factors. In these planula stolons instead of hydranth-stem axes develop because in the earlier stages of acclimation or recovery a considerable degree of inhibition is still present. The apical region of the stolon axis is



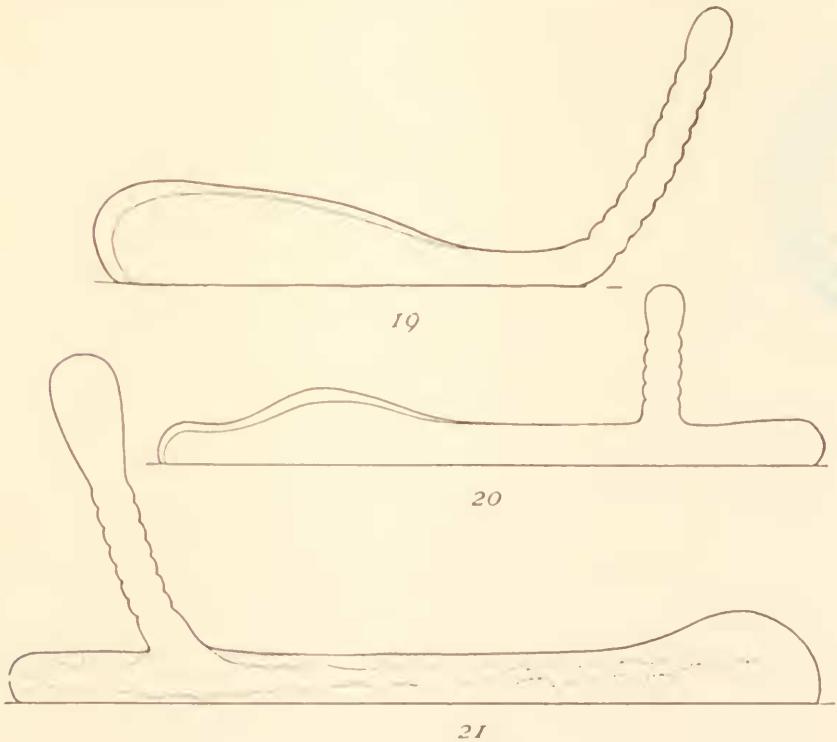
sufficiently active in relation to other levels to grow at their expense, but for the development of a hydranth-stem axis a higher metabolic rate is evidently necessary than for a stolon axis.

Figure 16 represents a larva in which the second gradient at the basal end did not develop before inhibition occurred, or was obliterated by the inhibition, while the original gradient was not wholly obliterated. In Fig. 17 and probably in Fig. 18 both gradients have persisted through the inhibiting action and both develop independently as stolons.

The further history of these stolon outgrowths depends on the degree of acclimation or recovery. When a certain degree of inhibition persists, *e.g.*, very commonly in acclimation, in which the material remains exposed to the agent, the stolon tips

continue to grow at the expense of the more basal levels, which are gradually resorbed. Such growth may continue for weeks and extend over many centimeters, being ended only by exhaustion of nutritive material. It is discussed more fully in a later section.

When the degree of acclimation or recovery is such that the gradients attain or approach their normal physiological condition, hydranth-stem structures develop. In differential acclimation and recovery this condition is usually attained only gradually, consequently the first outgrowths are almost invariably stolons and these transform later into hydranth-stem axes as in Fig. 19,



or give rise by budding to such axes as in Figs. 20 and 21. In these figures the animals are viewed from the side, the original larval body and the stolons are in contact with the bottom and the hydranth-stem axes are more or less erect.

With the appearance of hydranth-stem axes the further growth

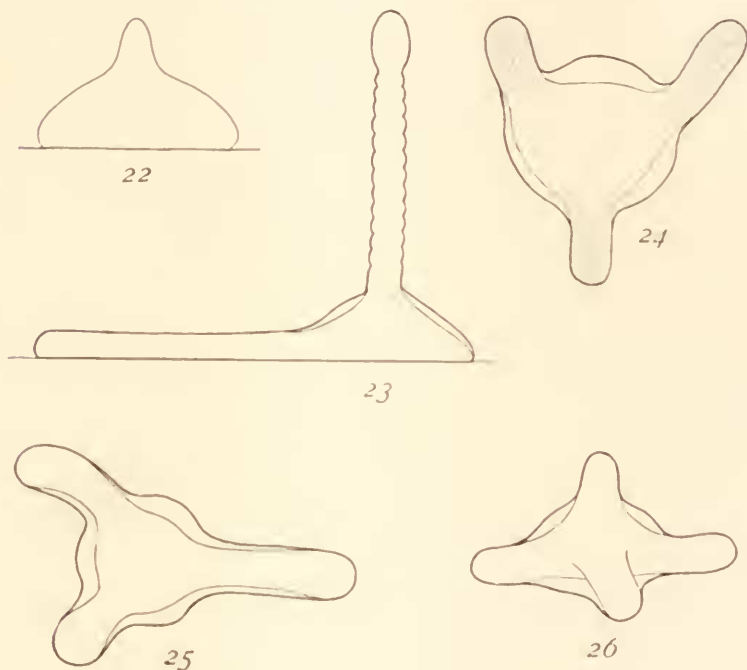
of the stolon is usually inhibited or retarded until the hydranth-stem axis attains sufficient length to permit some degree of physiological isolation of the stolon tip. Often the stolon serves as nutritive material for the developing hydranth and stem and may be completely resorbed. Fig. 19 shows a case in which such resorption is going on. The stolon, originally filling the perisarc, is much reduced and irregular in outline, while in the hydranth-stem axis the cœnosarc fills the perisarc. Moreover, the stolon cœnosarc, particularly in the most reduced regions, is almost transparent, while that of the hydranth-stem axis appears more granular and denser. By means of inhibiting conditions these hydranth-stem axes may be again transformed into stolon axes or inhibited to such an extent that they serve as material for the growth of stolon axes and are resorbed. With the approach of exhaustion also, the hydranth-stem axes usually undergo resorption, while the stolon axes persist and grow, at least for some days longer.

#### THE APPEARANCE OF NEW POLARITIES.

As noted above, the spherical, apparently completely apolar forms undergo no further development unless some degree of acclimation or recovery occurs and then they apparently develop new polarities. Occasionally in cases of rapid recovery a hydranth-stem axis arises directly from the upper free surface of the flattened mass (Fig. 22). In the absence of landmarks the possibility cannot be absolutely excluded that this outgrowth represents the hydranth-stem axis of normal development. On the other hand, observation indicates that the spherical forms may come to rest with any part of their surface in contact and that their polarity has been completely obliterated. If this is true, the hydranth-stem axis developing from the upper surface represents a new polarity determined by differential exposure of free surface and surface in contact, probably involving differences in intake of oxygen or the giving off of  $\text{CO}_2$  or both.

Figure 23 shows in side view a case of simultaneous development of two axes from a spherical apolar form. The axis arising from the upper surface is a hydranth-stem axis, that from the side, a stolon axis. Since these axes develop from a spherical, apparently apolar form, it seems probable that both represent

new polarities, the hydranth-stem axis being determined as a gradient by the greater respiratory activity occurring on the upper free surface, the stolon axis by some local region of greater activity on the margin.



Usually, however, acclimation or recovery occurs so slowly that the outgrowths which arise from these spherical forms are at first stolons and only later transform into, or give rise to hydranth-stem axes. In many of these cases there can be no doubt that new gradients or axes originate since three and not infrequently four stolons may arise from a single individual (Figs. 24-26, all viewed from above). These axes, or at least some of them, are evidently "adventitious," *i.e.*, they are determined by slight local chance differences among the cells, each stolon originating as a localized region in which the cellular activity is greater than in surrounding regions. This region begins to grow at the expense of other parts about it and as it grows the gradation in activity, which is at first about a center becomes an axial gradient as growth proceeds. Each outgrowing stolon is such a gradient

with high end at the tip. In Fig. 24 the basipetal gradient of cytolysis in lethal concentrations is indicated in the three stolons.

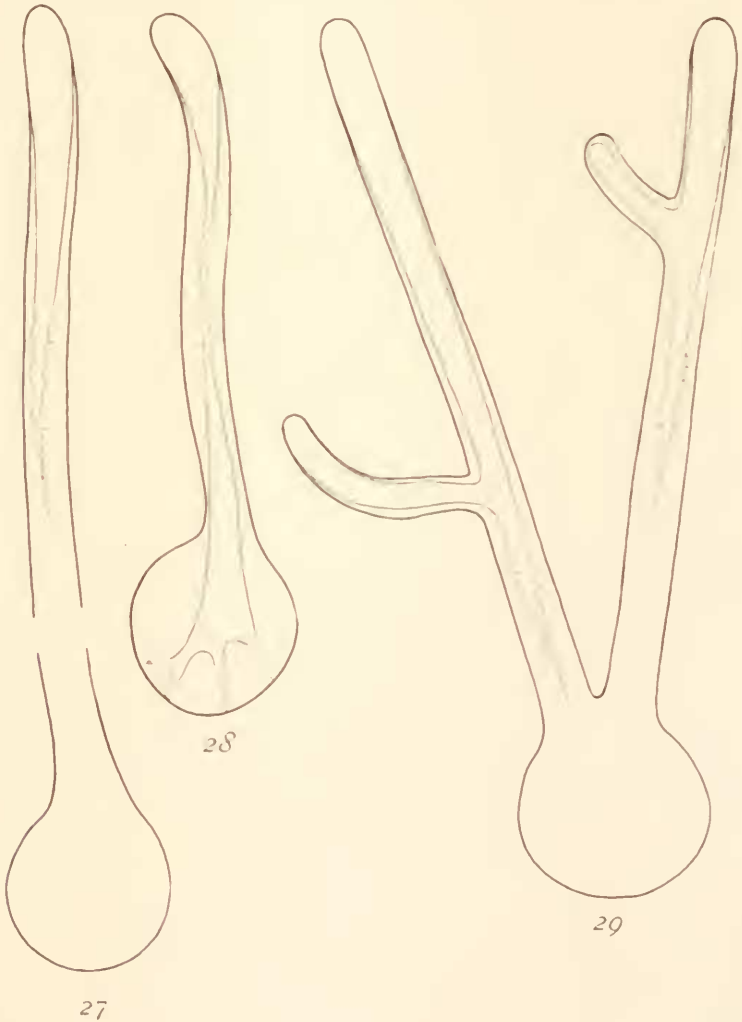
When acclimation or recovery proceeds far enough, the tips of such stolons sooner or later transform into hydranth-stem axes, like the stolon of Fig. 19, or hydranth-stem axes arise as adventitious buds from their upper surfaces, as in Figs. 20 and 21. In this way an individual which has been made apolar through differential inhibition may develop during acclimation or recovery several new stolon axes and each of these may later give rise to several hydranth-stem axes. On the other hand, if acclimation or recovery is only partial each stolon may continue to grow indefinitely as a stolon until exhaustion occurs.

#### THE GROWTH AND SEPARATION OF STOLONS.

The continued growth of stolons under slightly inhibiting conditions shows certain features of interest, some of which were briefly described in an earlier paper (Child, '23) as observed in stolons arising from hydroid colonies in consequence of inhibiting conditions. The stolons arising from the inhibited developmental stages grow at the expense of other parts and in time the whole substance of the inhibited larva may be used in the growth of the stolon or stolons arising from it, leaving only the empty perisarc in the position of the larval body and an empty perisarc tube connecting this with the stolon. Fig. 27 shows a case in which an inhibited form gave rise to a single stolon, which finally used up the whole larval body in its growth. At the stage shown the tip of the stolon had reached a point several centimeters distant from its point of origin, only a small part of the perisarc tube connecting stolon and larval body being shown in the figure. After using the substance of the larval body, the stolon tip continues to grow at the expense of its more basal levels. The cells of the tip remain in good condition, while those of the basal end become shrunken and transparent and undergo atrophy. In this way the *cœnosarc* of the stolon becomes shorter and shorter until only a small mass of tissue remains.

Figure 29 shows a case in which an inhibited form gave rise to two stolons near together. At the stage shown they have used the whole larval body and then their own substance to such an extent that they are entirely separate from each other, except for

the perisarcal tubes, and each has branched. In the later history of this case the cœnosarc of the larger lateral branch and that of the main stolon from which it arose became separated and each pursued its own course, the tip continuing to grow at the expense of more basal regions. The shorter lateral branch on the other main stolon is undergoing resorption at the stage figured and later was completely resorbed. Occasionally a growing stolon leaves a part of the larval body behind as in Fig. 28. This



apparently occurs when the stolon is not very active and the gradient is therefore short. Under such conditions the tip is apparently able to obtain nutrition only from regions within a short distance. The region in which atrophy occurs evidently represents approximately the limit of this distance.

In the earlier stages of this process of growth during starvation the development of hydranth-stem axes from such stolons may be induced by providing optimal conditions, but in later stages this transformation occurs less frequently and with the approach of exhaustion, but before stolon growth ceases, all attempts to induce transformation have thus far failed.

This continued growth of the stolon tip at the expense of the larval body and later of its own substance is an interesting case of a more active region maintaining itself at the expense of less active regions. Whether the atrophy of the less active regions represents primarily a more rapid autolysis or some other process, it is clearly evident that the most active region, the high end of the stolon gradient, succeeds in using the substance of all other levels, not only for maintenance, but for growth. Such stolons may grow over a distance of 10-15 centimeters before exhaustion occurs. Doubtless each stolon level lives at the expense of levels below it and sooner or later becomes a source of nutrition to levels above it in the gradient. This relation may mean simply that less active regions undergo autolysis more rapidly and that the more active regions are able because of their activity to use the products of autolysis in maintenance and even in growth.

A similar relation between different levels of the axial gradient appears in other cases of reduction by starvation. For example, in planarians and other forms undergoing reduction the proportions approach those of younger animals because the higher levels of the polar gradient, particularly the head region, maintain themselves to some extent at the expense of lower levels and so undergo reduction less rapidly than those.

#### EXPERIMENTAL RECORDS.

The uniformity of result makes it entirely unnecessary to give the records of all experiments in full. A few characteristic records are given in Table I. as examples. Other records differ from these only as these differ from each other, *i.e.*, in degree and



rate of differential inhibition, acclimation or recovery, according to the conditions of the experiment. The various forms, normal and modified, are indicated in Table I. by abbreviations as follows:

- B.* Normal blastula (Figs. 1, 2).
- IB.* Differentially inhibited blastula (Figs. 4-9).
- Ap.* Spherical or flattened apolar form (Figs. 10-13).
- EP.* Early planula (Fig. 3).
- LP.* Late planula, elongated (Fig. 14).
- IP.* Differentially inhibited planula, shorter, thicker than normal, with excess of entoderm.
- II.* Hydranth-stem axis developing directly from basal end of planula attached by apical end; "normal" hydroid development (Fig. 15).
- III.* Hydranth-stem axis developing directly from apolar form (Figs. 22, 23).
- SIH.* Hydranth-stem axis developing indirectly by transformation of, or budding from a stolon axis (Figs. 19-21).
- St.* Stolon axis (Figs. 16-18, 24-29).
- Dd.* Dead.

Each series of Table I. consisted of one hundred or more eggs or early embryos. All different forms observed were recorded at each examination and many drawings were made. In the table the relative frequency of the different forms is roughly indicated by enclosing in parentheses the designations of those forms which were present only in relatively small numbers (approximately 20 per cent. or less). The designations not so enclosed represent the characteristic forms of the series at the time indicated. As indicated at various points in the table the solutions were sometimes diluted after inhibition had occurred, particularly when the appearance of the material indicated that development was not likely to proceed farther in the concentration originally used. Such dilution made acclimation possible or hastened it. The series given are characteristic. Frequent repetitions during the same and different summers showed nothing essentially different from the data presented.

Taking Series 4 I. as an example, the Table shows that in KCN,  $m/25,000$  the forms after one day were inhibited blastulae and

TABLE I.

Series.	Controls	2	4 I	4 II	3 I	3 II	9	8 III	5
Agent	water	KCN	KCN	KCN	HCl	water 2 days HCl	LiCl	LiCl	meduse 2 days
Concentration		m/20,000	m/25,000	m/50,000	pH 7.4+	pH 7.2	m/80	m/160	pH decreased to 7.5
Days after beginning of experiment									
1	B EP	(B) IB Ap	(B) IB Ap	EP IP	IP Ap		IB (Ap) diluted 3/4	IB Ap	IB
2						EP (LP)	(IP) Ap	IP Ap	(EP) IP (Ap) meduse re- moved; water
3		IP Ap returned to water	(IP) Ap	LP IP (Ap)	St Ap			IP Ap (St)	
4	H (StH LP)	(IP) Ap Dd	(IP) Ap diluted 1/3		St (StH Ap) diluted 1/3	(LP) St Ap diluted 2/3		(StH) St Ap Dd	IP Ap
5		(IP Ap) Dd	(IP) Ap	H StH St	StH St	St (Ap)			IP Ap
6	H (StH)								St (Ap)
8									(StH) St
9			(StH) St Ap				StH St Ap		
12			NH StH (St Ap)						

apolar forms with a few normal blastulae; after four days chiefly apolar forms with a few inhibited planulae; solution now diluted one third; after nine days chiefly outgrowing stolon axes and apolar forms with a few stolon axes giving rise to hydranths; after twelve days chiefly new hydranth-stem axes arising directly from previously apolar forms and hydranth-stem axes arising from stolon axes, with a few stolon axes bearing no hydranths and a few apolar forms still remaining.

#### MODIFICATION OF DEVELOPMENT IN *Gonothyrea*.

A few experiments with HCl and KCN, exposure beginning with the early planula after emergence from the gonophore, were sufficient to show that the modifications resulting from differential inhibition and from differential acclimation and recovery are the same in *Gonothyrea* as in *Phialidium*. To decrease in pH the susceptibility of *Gonothyrea* is less than that of *Phialidium*. For example, HCl, pH 7.4 has very little inhibiting effect on *Gonothyrea*, the planulae usually attaching in the normal manner and giving rise to hydranth-stem axes without stolon outgrowths. Series 3 I. of Table I. shows that this pH is strongly inhibitory for *Phialidium*. For obliteration of polarity in *Gonothyrea* a concentration of HCl giving pH 7-6.8 was found to be necessary and as acclimation occurred, stolon axes developed and even gave rise to hydranths. As might be expected from these results crowding with the medusae had little or no effect on development within the range used for *Phialidium*.

#### DISCUSSION.

The differentially inhibiting effect of the agents on the blastula and early planula stages is sufficiently evident. In the normal blastula the region of basal thickening and of immigration represents the low end of the apico-basal gradient (Child, '25) and the effect of the agents is to bring higher levels of the gradient into a condition in which they behave like the basal region. In the extreme cases (Figs. 10-12) in which polarity is apparently obliterated, the whole blastula wall, including even the apical pole, behaves like the basal region. Taking this behavior as a criterion, it appears to be true that the whole blastula is reduced to the physiological level of the basal region. Evidently such a

change in condition is differential with respect to the axis: the apical region is most, the basal region least affected. When this reduction of all regions to the same physiological level is complete, the physiological gradient is obliterated and the fact that such forms show no indications of polarity until new gradients are determined in them is additional evidence for the conclusion that the gradient constitutes the polarity.

The fact that the basal region usually shows growth and thickening far in excess of the normal may seem at first glance to conflict with the interpretation of these modifications in terms of differential inhibition, but, as a matter of fact such overgrowth is a consequence of the differential inhibition. The basal region is less inhibited than the apical, consequently it is more active relatively to the apical region in the inhibited than in the normal forms. This relatively greater activity enables it to obtain a larger proportion of the available nutritive substance and so to grow to a larger size, though more slowly than in normal development. Up to a certain degree the excess and extension of basal thickening increases with the decrease in apical, as compared with basal activity. But with more extreme degrees of differential inhibition all levels become more and more alike and more basal as regards physiological condition and differences of growth and development disappear, as in the spherical apolar forms. The complete disappearance of these differences and the reduction of the larva to a spherical apolar condition are more likely to occur when the inhibiting factor has acted from the early stages of development and has obliterated the gradient before the differences in behavior of ectodermal and entodermal regions have been established.

As pointed out above, actual reversal of the physiological gradient occurs in some cases, the reversal being indicated by reversal of orientation in locomotion and reversed susceptibility and reduction gradients. In such cases the differential inhibiting action of the agent is such that the original apical region is reduced to a lower level of activity than the original basal region. Since such reversal occurs only after basal thickening has begun, it may result in a great increase of that thickening. The cases of emigration of cells from the basal region (Fig. 6) suggest that the polarity of these cells, *i.e.*, the differences between the exterior

and the interior pole, have also undergone reversal, so that instead of immigrating into the blastocœl the cells emigrate.

It may be noted in passing that the less extreme degrees of differential inhibition (Figs. 5-9) show interesting resemblances to the exogastrulae and related forms such as solid blastulae of the echinoderms, which also result from differential inhibition (MacArthur, '24). In both the hydrozoan and echinoderm modifications the entoderm—and in echinoderms the mesenchyme—is greatly increased at the expense of ectoderm, because the lower levels of the gradient are normally less active and therefore less susceptible and less inhibited than the higher levels. Actual reversal of the susceptibility gradient may also occur in both hydrozoan and echinoderm. The cases of emigration of cells (Fig. 6) correspond most closely to the exogastrulae.

Spek ('18) has maintained that gastrulation is due to difference in the degree of swelling of internal and external parts of the cells concerned and that lithium salts and some other agents determine exogastrulation by reversing these differences. Whether or not this interpretation be correct, the differential action of the agents shows clearly the same relation to the gradient in both hydrozoan and echinoderm.

That the inhibition, even though it does not obliterate polarity, interferes with the usual sequence of events in later stages is shown by the fact that when acclimation or recovery occurs, the outgrowth may be apical (Fig. 16) or both apical and basal (Fig. 17), that is, the stolon may arise from the primary gradient or from both primary and secondary gradient and probably also from the secondary alone (Fig. 19). These differences undoubtedly depend on differences in stage of development at time of exposure and on degree of inhibition. The development of both apical and basal stolons indicates that under the inhibiting conditions the two ends have become independent of each other.

The conditions determining the "adventitious" polarities in the acclimation and recovery of apolar forms (Figs. 24-26) are obviously accidental for no two individuals are alike as regards localization and time of appearance of the different axes. Evidently these axes originate as local areas of slightly greater activity in some region or regions of the mass. Some of them may represent persisting traces of the original polarity or

polarities, but some of them are certainly, and all of them may be new axes. The fact is of interest that in such forms adventitious stolon-axes may arise from any part of the free surface, but the direct origin of a hydranth-stem axis has been observed only from the most exposed portion farthest from the surface in contact (Figs. 22, 23).

The fact that the stolon axes usually appear first in acclimation and recovery and only later transform into, or give rise by budding to hydranth-stem axes confirms the conclusion of an earlier paper (Child, '23). In that paper it was shown that apical regions of various hydroid colonies can be transformed into stolon axes by slight degrees of inhibition or depression and that the stolons can again give rise to hydranth-stem axes with acclimation to, or recovery from the action of the agent. Such apical transformations do not represent reversals of polarity, but rather simply a depression of the gradient to a lower physiological level. In Lund's experiments on *Obelia* with electric current (Lund, '21) the position of the piece with respect to the electrodes merely determines whether the gradient arising at a given end shall be more or less inhibited and develop as a stolon or whether it shall attain the higher physiological levels characteristic of the hydranth-stem axis. Since axes, either stolon or hydranth-stem, very commonly arise from both ends of pieces without action of the electric current, it is evident that the current is not necessary for the determination of these polarities, even though it does determine their character as stolon or hydranth-stem axes and may, when acting, assist in determining the gradient at one end or the other of the piece. In any case the effects of the current are in no way specific as regards determination of the two sorts of axes, for, as I have shown, essentially the same results can be obtained with various chemical and physical agents in both hydroid colonies and developmental stages. Apparently all that is necessary to transform a hydranth-stem gradient into a stolon-gradient is a slight degree of inhibition, and the reverse transformation is accomplished by environmental changes in the reverse direction. In Lund's experiments with *Obelia* the anode apparently acts to some extent as an accelerating, the cathode as an inhibiting agent.

Physiologically the stolon does not represent simply the basal

end of the hydranth-stem axis, but is itself an axis, a gradient, as has been demonstrated in various ways. Like new hydranth-stem axes, it originates from a bud and the high end of its gradient constitutes a growing tip instead of a hydranth. At the stolon tip synthesis of protoplasm, growth and cell division apparently keep pace with each other as in the growing tip of a plant, so that differentiation does not occur, but when the tip is raised to a higher physiological level by change in external conditions it loses the capacity for unlimited growth and differentiates sooner or later into a hydranth.

The demonstration that the stolon is a somewhat inhibited axis or gradient throws light on many otherwise puzzling features of the behavior of isolated pieces of hydroids and hydroid colonies. The stolon gradient may be inhibited, either by external conditions or by its nearness to a dominant hydranth region (Child, '15, pp. 91-2). Consequently a stolon axis may arise at a cut surface which is near vigorous hydranth regions and it may also arise under conditions which prevent formation or maintenance of hydranths. Pieces in deep standing water may produce stolons, those in shallow, or frequently changed water hydranth-stem axes. Pieces may produce first stolons, then hydranth-stem axes or *vice versa*, with slight differences in conditions of culture.

Three conclusions based on the evidence presented in this and earlier papers of this series and on the work of Lund and others are important in this connection: first, the stolon as well as the hydranth-stem represents an axis, a polarity, a gradient; second, the stolon gradient represents primarily lower levels of physiological activity than the hydranth-stem gradient and reversible transformation in either direction is possible and does not necessarily involve reversal of polarity; third, hydranth-stem or stolon axis may be determined according to conditions by the quantitative differential or local action of environmental factors independently of their specific constitution. These conclusions afford a physiological basis for the interpretation of data at hand and for further investigation and control of polarity.



## SUMMARY.

1. Differential inhibition of planula development can be brought about in the hydrozoan, *Phialidium gregarium*, by KCN, LiCl, ethyl urethane HCl (probably CO<sub>2</sub>) and by the presence of the medusæ (CO<sub>2</sub>). Resulting modifications range from slight increase and extension of basal thickening of blastula wall followed by development of slightly modified planulae to spherical, solid, apparently apolar forms. In these immigration and mass ingrowth are increased and take place from all parts of the wall, and further development occurs only if a new polarity arises.

2. In differential acclimation in low concentrations of inhibiting agents and differential recovery after return to water further development of persisting axes may occur or new polarities may arise through differential exposure of the surfaces of apolar forms or through "adventitious" localization of regions of greater activity. In rapid acclimation or recovery such axes may develop directly as hydranth-stem axes, but usually they are at first still more or less inhibited and develop as stolons and their tips transform later into hydranth-stem axes, or such axes arise by budding from their free surfaces.

3. The stolon represents a polarity, a gradient, at a lower level of physiological activity than the hydranth-stem axis. Transformation of hydranth-stem into stolon results from inhibition, of stolon into hydranth-stem from acceleration; neither involves necessarily a reversal of polarity.

## REFERENCES.

Child, C. M.

'15 Individuality in Organisms. Chicago.

'23 The Axial Gradients in Hydrozoa. V. BIOL. BULL., XLV.

'24 Physiological Foundations of Behavior. New York.

'25 The Axial Gradients in Hydrozoa. VI. BIOL. BULL., XLVIII.

Lund, E. J.

'21 Experimental Control of Organic Polarity by the Electric Current. I. Jour. Exp. Zool., XXXIV.

MacArthur, J. W.

'24 An Experimental Study and a Physiological Interpretation of Exogastrulation and Related Modifications in Echinoderm Embryos. BIOL. BULL., XLVI.

**Metschnikoff, E.**

'86 Embryologische Studien an Medusen. Wien.

**Smith, H. W. and Clowes, G. H. A.**

'24 The Influence of Carbon Dioxide on the Velocity of Division of Marine Eggs. Amer. Jour. Physiol., LXVIII.

**Spek, J.**

'18 Differenzen im Quellungs-zustand der Plasmakolloide als eine Ursache der Gastrulainvagination sowie der Einstülpungen und Faltungen der Zellplatten überhaupt. Kolloid-chem. Beihefte, IX.